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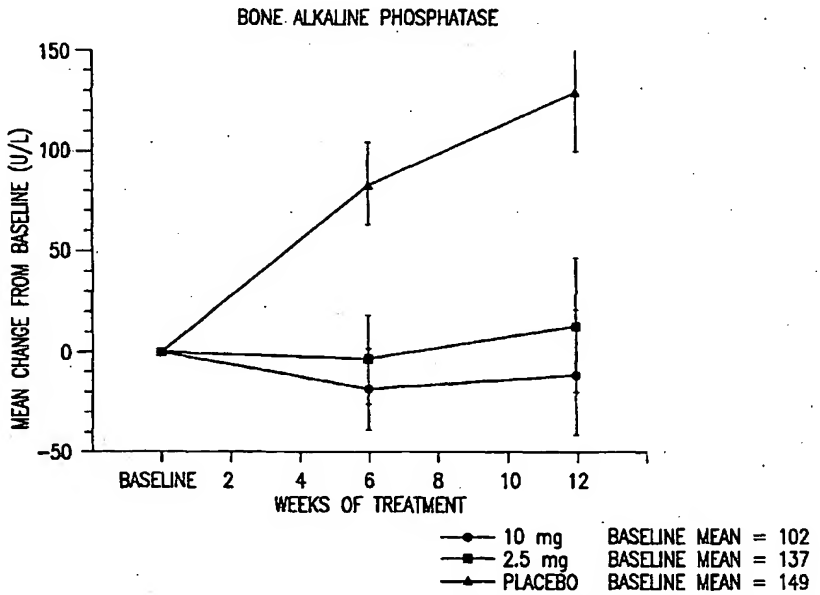
(54) Title: METHODS OF TREATING CANCER AND THE PAIN ASSOCIATED THEREWITH USING ENDOTHELIN ANTAGONISTS



(57) Abstract: The instant invention is directed to methods for the inhibition of bone metastases, methods for the prevention of growth of new metastases, methods for the inhibition of bone turnover, and methods for the prevention of bone loss in patients, including cancer patients, using an endothelin ET-A receptor antagonist.

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Methods of Treating Cancer And The Pain Associated Therewith Using Endothelin Antagonists

Field of the Invention

The instant invention is directed to methods for the inhibition of bone metastases, methods for the prevention of growth of new metastases, methods for the inhibition of bone turnover, and methods for the prevention of bone loss in patients, including cancer patients, using an endothelin ET-A receptor antagonist.

Background of the Invention

Endothelin (ET), a 21 amino acid peptide, is produced by enzymatic cleavage of a precursor peptide by an endothelin converting enzyme. First discovered in vascular endothelial cells, ET and ET/ET receptor binding are now known to modulate smooth muscle tone, blood flow, cell proliferation and differentation, protein synthesis, and metabolic function in a variety of tissues and cell types such as ovary, prostate, skin, and brain.

ET/ET receptor binding has been shown to constrict arteries and veins; increase mean arterial blood pressure; decrease incardiac output; increase cardiac contractility in vitro; stimulate mitogenesis in vascular smooth muscle cells in vitro; contract non-vascular smooth muscle such as guinea pig trachea, human urinary bladder strips and rat uterus in vitro; increase airway resistance in vivo; induce formation of gastric ulcers; stimulate release of atrial natriuretic factor in vitro and in vivo; increase plasma levels of vasopressin, aldosterone, and catecholamines; inhibit release of renin in vitro; and stimulate release of gonadotropins in vitro.

ET/ET receptor binding also causes vasoconstriction on vascular smooth muscle (Nature 332 411 (1988), FEBS Letters 231 440 (1988) and Biochem. Biophys. Res. Commun. 154 868 (1988)). In fact, an anti-ET antibody has been shown to ameliorate adverse effects of renal ischemia on renal vascular resistance and glomerular filtration rate (J. Clin. Invest. 83 1762 (1989)). In addition, an anti-ET antibody attenuated both the nephrotoxic effects of intravenously administered cyclosporin (Kidney Int. 37 1487 (1990)) and the infarct size in a coronary artery ligation-induced myocardial infarction model (Nature 344 114 (1990)).

A nonpeptide ET antagonist prevents post-ischaemic renal vasoconstriction in rats, prevents the decrease in cerebral blood flow due to subarachnoid hemorrhage in rats, and decreases MAP in sodium-depleted squirrel monkeys when dosed orally (Nature 365: 759-761 (1993)). A similar effect of an ET antagonist on arterial calibera has also been recently reported (Biochem. Biophys. Res. Comm., 195: 969-75 (1993).

An ET receptor antagonist reduced injury in a rat model of colitis (EUR. J. Pharmacol. 1996, 309, 261-269) and prevented ischemia-reperfusion injury in kidney transplantation (Transplant Int 1996, 9, 201-207). The use of ET antagonists in the treatment of angina, pulmonary hypertension, Raynaud's disease, and migraine has also been suggested (Drugs 1996, 51,12-27). In malignant growth disorders, ET and its growth-promoting effects have been best characterized in prostate cancer, (Nature Medicine 1995, 1, 944-949) wherein ET acts as a modulator in osteoblastic bone lesion (UROLOGY 53:1063-1069, 1999).

Given the results from these and other reports which illuminate the role of ET/ET receptor binding in disease states, and the knowledge that blocking ET/ET receptor binding results in improvement or reversal of endothelin-induced

disease states, agents which antagonize ET/ET receptor binding activity, designated as ET receptor antagonists, can provide substantial benefit in many therapeutic areas.

Summary of the Invention

In one embodiment of the instant invention, therefore, is disclosed a method for inhibiting bone metastases in a patient which comprises administering to the patient in need thereof a therapeutically effective amount of an endothelin ET-A receptor antagonist.

In another embodiment of the invention is disclosed a method for preventing new bone metastases in a patient which comprises administering to the patient in need thereof a therapeutically effective amount of an endothelin ET-A receptor antagonist.

In another embodiment of the instant invention, therefore, is disclosed a method for inhibiting metastatic growth in a patient which comprises administering to the patient in need thereof a therapeutically effective amount of an endothelin ET-A receptor antagonist.

In another embodiment of the invention is disclosed a method for inhibiting bone loss in a patient which comprises

administering to the patient in need thereof a therapeutically effective amount of an endothelin ET-A receptor antagonist.

In another embodiment of the instant invention, is disclosed a method for inhibiting bone turnover in a patient which comprises administering to the patient in need thereof a therapeutically effective amount of an endothelin ET-A receptor antagonist.

In another embodiment of the invention is disclosed a method for the reduction of cancer related pain in a patient in need thereof which comprises administering to the patient a therapeutically effective amount of an endothelin ET-A receptor antagonist.

In another embodiment of the instant invention is disclosed therapeutically acceptable formulations of an endothelin ET-A receptor antagonist, optionally in the presence of a co-therapeutic agent, for use in these methods.

Brief Description of the Drawings

Figure 1 illustrates levels of interleukin-6 (IL-6) in a subject population treated with a placebo or 2.5 mg or 10 mg ABT-627.

Figure 2 illustrates levels of prostate specific antigen (PSA) in a subject population treated with a placebo or 2.5 mg or 10 mg of ABT-627.

Figure 3 illustrates VAS score levels relating to pain assessment in a subject population treated with a placebo or 2.5 mg or 10 mg of ABT-627.

Figure 4 illustrates crosslinked N-telopeptides (degradation) in a subject population treated with a placebo or 10 mg ABT-627.

Figure 5 illustrates bone alkaline phosphatase (BAP) (formation) in a subject population treated with a placebo or 10 mg ABT-627.

Figure 6 illustrates skeletal involvement in a subject population treated with a placebo or 10 mg ABT-627.

Figure 7 illustrates acid phosphatase levels in a subject population treated with a placebo or 10 mg ABT-627.

Detailed Description of the Invention

Endothelin receptor antagonists are employed in the practice of the instant invention. Endothelins are a family of peptides mainly synthesized and released by endothelial cells. The term "endothelin" refers to a family of homologous 21-amino acid peptides found in 3 distinct isoforms: ET-1, ET-2, and ET-3.

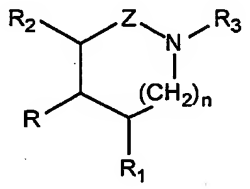
The term "endothelin ET-A receptor antagonist" includes both compounds which antagonize the ET-A receptor in a selective manner, as well as compounds which antagonize the ET-A receptor in a non-selective manner. An example of the latter type of compound would be a compound that antagonizes the ET-A receptor and also antagonizes the ET-B receptor.

The term "primary cancer" means cancer in a specific tissue, which is first in time or in order of development. Primary cancers include, but are not limited to, breast, prostate, lung, kidney, thyroid, brain, heart, intestine, ovary, myeloma, lymphoma, sarcoma, and osteosarcoma.

The term "cancer-related pain" includes pain which arises from direct invasion or expansion of a tumor into tissue, such as bone or nerve; pain which arises from the consequences of tumor invasion or expansion, such as bone collapse due to

cancer erosion or secretion of noxious agents which modulate or produce pain; and pain mediated by ischemia, i.e. reduced blood flow.

Specifically, a compound of formula I may be employed in the practice of the instant invention



I

wherein

R is $-(CH_2)_m-W$;

Z is selected from $-C(R_{18})(R_{19})-$ and $-C(O)-$;

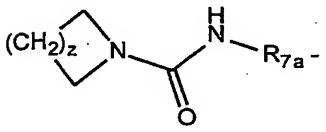
R_1 and R_2 are independently selected from hydrogen, loweralkyl, alkenyl, alkynyl, alkoxyalkyl, alkoxycarbonylalkyl, hydroxyalkyl, haloalkyl, haloalkoxyalkyl, alkoxyalkoxyalkyl, thioalkoxyalkoxyalkyl, cycloalkyl, cycloalkylalkyl, aminocarbonylalkyl, alkylaminocarbonylalkyl, dialkylaminocarbonylalkyl, aminocarbonylalkenyl, alkylaminocarbonylalkenyl, dialkylaminocarbonylalkenyl, hydroxyalkenyl, aryl, arylalkyl, aryloxyalkyl,

arylalkoxyalkyl, (N-alkanoyl-N-alkyl)aminoalkyl, alkylsulfonylamidoalkyl, heterocyclic, (heterocyclic)alkyl, and $(R_{aa})(R_{bb})N-R_{cc}-$,

with the proviso that one or both of R_1 and R_2 is other than hydrogen;

R_3 is selected from $R_4-C(O)-R_5-$, $R_4-R_{5a}-$, $R_4-C(O)-R_5-N(R_6)-$, $R_6-S(O)_2-R_7-$ $R_{26}-S(O)-R_{27}-$, $R_{22}-O-C(O)-R_{23}-$, loweralkyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, aryl, arylalkyl, aryloxyalkyl, heterocyclic, (heterocyclic)alkyl, alkoxyalkyl, alkoxyalkoxyalkyl, and $R_{13}-C(O)-CH(R_{14})-$;

R_4 and R_6 are independently selected from $(R_{11})(R_{12})N-$, loweralkyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, aryl, arylalkyl, heterocyclic, (heterocyclic)alkyl, alkoxyalkyl, hydroxyalkyl, haloalkyl, haloalkenyl, haloalkoxyalkyl, haloalkoxy, alkoxyhaloalkyl, alkylaminoalkyl, dialkylaminoalkyl, alkoxy, and



R_5 is selected from a covalent bond, alkylene,

alkenylene, $-N(R_{20})-R_8-$, $-R_{8a}-N(R_{20})-R_8-$, $-O-R_9-$, and $-R_{9a}-O-R_9-$;

R_6 is selected from loweralkyl, haloalkyl, alkoxyalkyl, haloalkoxyalkyl, aryl or arylalkyl;

R_7 is a covalent bond, alkylene, alkenylene $-N(R_{21})-R_{10}-$, and $-R_{10a}-N(R_{21})-R_{10}-$;

R_8 is selected from alkylene and alkenylene;

R_9 is alkylene;

R_{10} is selected from alkylene and alkenylene;

R_{11} and R_{12} are independently selected from hydrogen, loweralkyl, haloalkyl, alkoxyalkyl, haloalkoxyalkylalkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, aryl, heterocyclic, arylalkyl, (heterocyclic)alkyl, hydroxyalkyl, alkoxy, aminoalkyl,trialkylaminoalkyl, alkylaminoalkyl, dialkylaminoalkyl, and carboxyalkyl;

R_{13} is selected from amino, alkylamino and dialkylamino;

R_{14} is selected from aryl and $R_{15}-C(O)-$;

R_{15} is selected from amino, alkylamino and dialkylamino;

R_{16} is selected from loweralkyl, haloalkyl, aryl and dialkylamino;

R_{17} is loweralkyl;

R_{18} and R_{19} are independently selected from hydrogen and loweralkyl;

R_{20} is selected from hydrogen, loweralkyl, alkenyl, haloalkyl, alkoxyalkyl, haloalkoxyalkyl, cylcoalkyl and cycloalkylalkyl;

R_{21} is selected from hydrogen, loweralkyl, alkenyl, haloalkyl, alkoxyalkyl, haloalkoxyalkyl, aryl and arylalkyl;

R_{22} is selected from a carboxy protecting group and heterocyclic;

R_{23} is selected from covalent bond, alkylene, alkenylene and $-N(R_{24})-R_{25}-$;

R_{24} is selected from hydrogen and loweralkyl;

R_{25} is alkylene;

R_{26} is selected from loweralkyl, haloalkyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, aryl, arylalkyl, heterocyclic, (heterocyclic)alkyl, alkoxyalkyl and alkoxy-substituted haloalkyl;

R_{27} is selected from alkylene and alkenylene;

R_{5a} is selected from alkylene and alkenylene;

R_{7a} is alkylene;

R_{8a} is selected from alkylene and alkenylene;

R_{9a} is alkylene;

R_{10a} is selected from alkylene and alkenylene;

R_{aa} is selected from aryl and arylalkyl;

R_{bb} is selected from hydrogen and alkanoyl;

R_{cc} is alkylene;

m is 0-6;

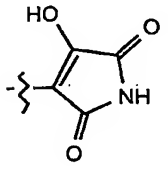
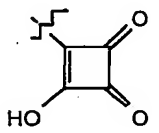
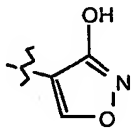
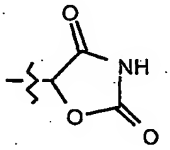
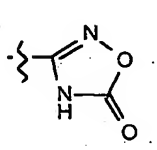
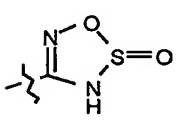
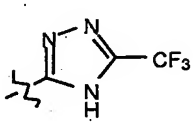
n is 0 or 1;

z is 0-5;

E is selected from hydrogen, loweralkyl and arylalkyl;

G is selected from hydrogen and a carboxy protecting group; and

W is selected from $-C(O)_2-G$; $-PO_3H_2$, $-P(O)(OH)(E)$, -CN, $-C(O)NHR_{17}$, alkylaminocarbonyl, dialkylaminocarbonyl, tetrazolyl, hydroxy, alkoxy, sulfonamido, $-C(O)NHS(O)_2R_{16}$, $-S(O)_2NHC(O)R_{16}$,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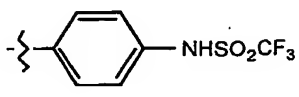
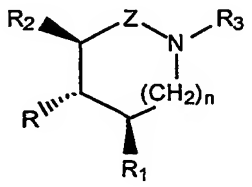


or a pharmaceutically acceptable salt thereof.

A preferred embodiment of the a compound of formula I is a compound of formula II



II

wherein the substituents $-R_2$, -R and $-R_1$ exist in a *trans,trans* relationship and Z, n, R, R_1, R_2, and R_3 are as defined above.

Compounds of formulas I and II are endothelin antagonists, specifically ET_A-selective endothelin antagonists.

Another preferred embodiment of the invention is a compound of formula I or II wherein n is 0 and Z is $-CH_2-$.

Another preferred embodiment of the invention is a compound of formula I or II wherein n is 1 and Z is $-CH_2-$.

Another preferred embodiment of the invention is a compound of formula I or II wherein n is 0, Z is $-CH_2-$, and R_3

is R_4-C(O)-R_5- , R_6-S(O)$_2$-R_7- or R_{26}-S(O)-R_{27}- wherein R_4, R_5, R_6, R_7, R_{26} and R_{27} are as defined above.

Another preferred embodiment of the invention is a compound of formula I or II wherein n is 0, Z is -CH_2-, and R_3 is alkoxyalkyl or alkoxyalkoxyalkyl.

A more preferred embodiment of the invention is a compound of formula I or II wherein n is 0, Z is -CH_2-, and R_3 is R_4-C(O)-R_5- wherein R_4 is (R_{11})(R_{12})N- as defined above and R_5 is alkylene or R_3 is R_6-S(O)$_2$-R_7- or R_{26}-S(O)-R_{27}- wherein R_7 is alkylene, R_{27} is alkylene and R_6 and R_{26} are defined as above.

Another more preferred embodiment of the invention is a compound of formula I or II wherein n is 0, Z is -CH_2- and R_3 is R_4-C(O)-N(R_{20})-R_8- or R_6-S(O)$_2$-N(R_{21})-R_{10}- wherein R_8 and R_{10} are alkylene and R_4, R_6, R_{20} and R_{21} are defined as above.

An even more preferred embodiment of the invention is a compound of formula I or II wherein n is 0, R is tetrazolyl or -C(O)$_2$-G wherein G is hydrogen or a carboxy protecting group or R is tetrazolyl or R is -C(O)-NHS(O)$_2$$R_{16}$ wherein R_{16} is loweralkyl, haloalkyl or aryl,

Z is $-CH_2-$; R_1 and R_2 are independently selected from (i) loweralkyl, (ii) cycloalkyl, (iii) substituted aryl wherein aryl is phenyl substituted with one, two or three substituents independently selected from loweralkyl, alkoxy, halo, alkoxyalkoxy and carboxyalkoxy, (iv) substituted or unsubstituted heterocyclic, (v) alkenyl, (vi) heterocyclic (alkyl), (vii) arylalkyl, (viii) aryloxyalkyl, (ix) (N-alkanoyl-N-alkyl)aminoalkyl and (x) alkylsulfonylamidoalkyl, and R_3 is $R_4-C(O)-R_5-$ wherein R_4 is $(R_{11})(R_{12})N-$ wherein R_{11} and R_{12} are independently selected from loweralkyl, haloalkyl, alkoxyalkyl, haloalkoxyalkyl, aryl, arylalkyl, heterocyclic, hydroxyalkyl, alkoxy, aminoalkyl, and trialkylaminoalkyl, and R_5 is alkylene; or R_3 is $R_4-C(O)-N(R_{20})-R_8-$ or $R_6-S(O)_2-N(R_{21})-R_{10}-$ wherein R_4 is loweralkyl, aryl, alkoxy, alkylamino, aryloxy or arylalkoxy and R_6 is loweralkyl, haloalkyl, alkoxyalkyl, haloalkoxyalkyl, aryl or arylalkyl, R_8 and R_{10} are alkylene and R_{20} and R_{21} are loweralkyl; or R_3 is $R_6-S(O)_2-R_7-$ or $R_{26}-S(O)-R_{27}-$ wherein R_6 is loweralkyl or haloalkyl, R_7 is alkylene, R_{26} is loweralkyl and R_{27} is alkylene.

A yet more preferred embodiment of the invention is a

compound of formula I or II wherein n is 0, R is $-C(O)_2-G$ wherein G is hydrogen or a carboxy protecting group, tetrazolyl or $-C(O)-NHS(O)_2R_{16}$ wherein R_{16} is loweralkyl, haloalkyl or aryl, Z is $-CH_2-$, R_1 is (i) loweralkyl, (ii) alkenyl, (iii) alkoxyalkyl, (iv) cycloalkyl, (v) phenyl, (vi) pyridyl, (vii) furanyl, (viii) substituted or unsubstituted 4-methoxyphenyl, 4-fluorophenyl, 3-fluorophenyl, 4-ethoxyphenyl, 4-ethylphenyl, 4-methylphenyl, 4-trifluoromethylphenyl, 4-pentafluoroethylphenyl, 3-fluoro-4-methoxyphenyl, 3-fluoro-4-ethoxyphenyl, 2-fluorophenyl, 4-methoxymethoxyphenyl, 4-hydroxyphenyl, 4-t-butylphenyl, 1,3-benzodioxolyl, 1,4-benzodioxanyl or dihydrobenzofuranyl wherein the substituent is selected from alkoxy, alkoxyalkoxy and carboxyalkoxy, (ix) heterocyclic (alkyl), (x) arylalkyl, (xi) aryloxyalkyl, (xii) (N-alkanoyl-N-alkyl)aminoalkyl, or (xiii) alkylsulfonylamidoalkyl, R_2 is substituted or unsubstituted 1,3-benzodioxolyl, 7-methoxy-1,3-benzodioxolyl, 1,4-benzodioxanyl, 8-methoxy-1,4-benzodioxanyl, dihydrobenzofuranyl, benzofurnayl, 4-methoxyphenyl, dimethoxyphenyl, fluorophenyl or difluorophenyl and R_3 is $R_4-C(O)-N(R_{20})-R_8-$ or

R_6-$S(O)_2$-$N(R_{21})$-R_{10}- wherein R_8 and R_{10} are alkylene, R_{20} and R_{21} are loweralkyl, R_4 is loweralkyl, aryl, alkoxy, alkylamino, aryloxy or arylalkoxy and R_6 is loweralkyl, haloalkyl, alkoxyalkyl, aryl or arylalkyl.

Another yet more preferred embodiment of the invention is a compound of formula I or II wherein n is 0, R is $-C(O)_2-G$ wherein G is hydrogen or a carboxy protecting group, tetrazolyl or $-C(O)-NHS(O)_2R_{16}$ wherein R_{16} is loweralkyl, haloalkyl or aryl, Z is $-CH_2-$, R_1 is (i) loweralkyl, (ii) alkenyl, (iii) alkoxyalkyl, (iv) cycloalkyl, (v) phenyl, (vi) pyridyl, (vii) furanyl, (viii) substituted or unsubstituted 4-methoxyphenyl, 4-fluorophenyl, 3-fluorophenyl, 4-ethoxyphenyl, 4-ethylphenyl, 4-methylphenyl, 4-trifluoromethylphenyl, 4-pentafluoroethylphenyl, 3-fluoro-4-methoxyphenyl, 3-fluoro-4-ethoxyphenyl, 2-fluorophenyl, 4-methoxymethoxyphenyl, 4-hydroxyphenyl, 4-t-butylphenyl, 1,3-benzodioxolyl, 1,4-benzodioxanyl or dihydrobenzofuranyl wherein the substituent is selected from alkoxy, alkoxyalkoxy and carboxyalkoxy, (ix) heterocyclic (alkyl), (x) arylalkyl, (xi) aryloxyalkyl, (xii) (N-alkanoyl-N-alkyl)aminoalkyl, or (xiii) alkylsulfonylamidoalkyl, R_2 is substituted or unsubstituted

1,3-benzodioxolyl, 7-methoxy-1,3-benzodioxolyl, 1,4-benzodioxanyl, 8-methoxy-1,4-benzodioxanyl, dihydrobenzofuranyl, benzofurnayl, 4-methoxyphenyl, dimethoxyphenyl, fluorophenyl or difluorophenyl and R_3 is R_4-C(O)-R_5- wherein R_5 is alkylene and R_4 is $(R_{11})(R_{12})N$- wherein R_{11} and R_{12} are independently selected from loweralkyl, haloalkyl, alkoxyalkyl, haloalkoxyalkyl, aryl, arylalkyl, heterocyclic, hydroxyalkyl, alkoxy, aminoalkyl, and trialkylaminoalkyl.

Another yet more preferred embodiment of the invention is a compound of formula I or II wherein n is 0, R is $-C(O)_2$-G wherein G is hydrogen or a carboxy protecting group, tetrazolyl or $-C(O)-NHS(O)_2R_{16}$ wherein R_{16} is loweralkyl, haloalkyl or aryl, Z is $-CH_2-$, R_1 is (i) loweralkyl, (ii) alkenyl, (iii) heterocyclic(alkyl), (iv) aryloxyalkyl, (v) arylalkyl, (vi) aryl, (vii) (N-alkanoyl-N-alkyl)aminoalkyl, or (viii) alkylsulfonylamidoalkyl, R_2 is substituted or unsubstituted 1,3-benzodioxolyl, 7-methoxy-1,3-benzodioxolyl, 1,4-benzodioxanyl, 8-methoxy-1,4-benzodioxanyl, dihydrobenzofuranyl, benzofurnayl, 4-methoxyphenyl, dimethoxyphenyl, fluorophenyl or difluorophenyl wherein the

substituent is selected from loweralkyl, alkoxy and halogen and R_3 is R_4-C(O)-R_5- wherein R_5 is alkylene and R_4 is (R_{11})(R_{12})N- wherein R_{11} is loweralkyl and R_{12} is aryl, arylalkyl, hydroxyalkyl, alkoxy, aminoalkyl, trialkylaminoalkyl, or heterocyclic.

Another yet more preferred embodiment of the invention is a compound of formula I or II wherein n is 0, R is -$C(O)_2$-G wherein G is hydrogen or a carboxy protecting group, tetrazolyl or -C(O)-$NHS(O)_2R_{16}$ wherein R_{16} is loweralkyl, haloalkyl or aryl, Z is -CH_2-, R_1 is (i) loweralkyl, (ii) alkenyl, (iii) heterocyclic (alkyl), (iv) aryloxyalkyl, (v) arylalkyl, (vi) (N-alkanoyl-N-alkyl)aminoalkyl, or (vii) alkylsulfonylamidoalkyl,(vii) phenyl, or (ix) substituted or unsubstituted 4-methoxyphenyl, 3-fluoro-4-methoxyphenyl, 3-fluorophenyl, 3-fluoro-4-ethoxyphenyl, 2-fluorophenyl, 4-methoxymethoxyphenyl, 1,3-benzodioxolyl, 1,4-benzodioxanyl or dihydrobenzofuranyl wherein the substituent is selected from loweralkyl, haloalkyl, alkoxy, alkoxyalkoxy and carboxyalkoxy, R_2 is substituted or unsubstituted 1,3-benzodioxolyl, 7-methoxy-1,3-benzodioxolyl, 1,4-benzodioxanyl, 8-methoxy-1,4-benzodioxanyl, dihydrobenzofuranyl, 4-methoxyphenyl,

dimethoxyphenyl, fluorophenyl or difluorophenyl wherein the substituent is selected from loweralkyl, alkoxy and halogen and R_3 is R_6-S(O)$_2$-N(R_{21})-R_{10}- wherein R_{10} is alkylene, R_6 is loweralkyl, haloalkyl, alkoxyalkyl, haloalkoxyalkyl, aryl or arylalkyl and R_{21} is loweralkyl, haloalkyl, alkoxyalkyl, haloalkoxyalkyl, aryl or arylalkyl.

Another yet more preferred embodiment of the invention is a compound of formula I or II wherein n is 0, R is -C(O)$_2$-G wherein G is hydrogen or a carboxy protecting group, tetrazolyl or -C(O)-NHS(O)$_2$$R_{16}$ wherein R_{16} is loweralkyl, haloalkyl or aryl, Z is -CH_2-, R_1 is (i) substituted or unsubstituted 4-methoxyphenyl, 3-fluoro-4-methoxyphenyl, 3-fluorophenyl, 3-fluoro-4-ethoxyphenyl, 4-methoxymethoxyphenyl, 1,3-benzodioxolyl or 1,4-benzodioxanyl wherein the substituent is selected from loweralkyl, haloalkyl, alkoxy and alkoxyalkoxy, (ii) loweralkyl, (iii) alkenyl, (iv) heterocyclic (alkyl), (v) aryloxyalkyl, (vi) arylalkyl, (vii) (N-alkanoyl-N-alkyl)aminoalkyl, (viii) alkylsulfonylamidoalkyl,or (ix) phenyl, R_2 is substituted or unsubstituted 1,3-benzodioxolyl, 7-methoxy-1,3-benzodioxolyl, 1,4-benzodioxanyl, 8-methoxy-1,4-benzodioxanyl,

dihydrobenzofuranyl, 4-methoxyphenyl, dimethoxyphenyl, fluorophenyl or difluorophenyl wherein the substituent is selected from loweralkyl, alkoxy and halogen and R_3 is alkoxycarbonyl or R_6-S(O)$_2$-N(R_{21})-R_{10}- wherein R_{10} is alkylene, R_6 is loweralkyl, haloalkyl, alkoxyalkyl or haloalkoxyalkyl and R_{21} is loweralkyl, haloalkyl, alkoxyalkyl or haloalkoxyalkyl.

Another yet more preferred embodiment of the invention is a compound of formula I or II wherein n is 0, R is -C(O)$_2$-G wherein G is hydrogen or a carboxy protecting group, tetrazolyl or -C(O)-NHS(O)$_2$$R_{16}$ wherein R_{16} is loweralkyl or haloalkyl, Z is -CH_2-, R_1 is loweralkyl,alkenyl, heterocyclic (alkyl), aryloxyalkyl, aryalkyl, aryl, (N-alkanoyl-N-alkyl)aminoalkyl,, or alkylsulfonylamidoalkyl, and R_3 is R_4-C(O)-R_5- wherein R_5 is alkylene and R_4 is (R_{11})(R_{12})N- wherein R_{11} and R_{12} are independently selected from alkyl, aryl, hydroxyalkyl, alkoxy, aminoalkyl, trialkylaminoalkyl, and heterocyclic.

A still more preferred embodiment of the invention is a compound of formula I or II wherein n is 0, R is -C(O)$_2$-G wherein G is hydrogen or a carboxy protecting group,

tetrazolyl or -C(O)-NHS(O)$_2$R$_{16}$ wherein R$_{16}$ is loweralkyl or haloalkyl, Z is -CH$_2$-, R$_1$ is substituted or unsubstituted 4-methoxyphenyl, 4-fluorophenyl, 2-fluorophenyl, 4-methylphenyl, 4-trifluoromethylphenyl, 4-pentafluoroethylphenyl, 4-methoxymethoxyphenyl, 4-hydroxyphenyl, 4-ethylphenyl, 1,3-benzodioxolyl, 1,4-benzodioxanyl or dihydrobenzofuranyl wherein the substituent is selected from alkoxy, alkoxyalkoxy and carboxyalkoxy, (ii) loweralkyl, (iii) alkenyl, (iv) heterocyclic (alkyl), (v) aryloxyalkyl, (vi) arylalkyl, (vii) (N-alkanoyl-N-alkyl)aminoalkyl, (viii) alkylsulfonylamidoalkyl,or (ix) phenyl, R$_2$ is 1,3-benzodioxolyl, 1,4-benzodioxanyl, dihydrobenzofuranyl, benzofuranyl, 4-methoxyphenyl, dimethoxyphenyl, fluorophenyl or difluorophenyl and R$_3$ is R$_4$-C(O)-R$_5$- wherein R$_5$ is alkylene and R$_4$ is (R$_{11}$)(R$_{12}$)N- wherein R$_{11}$ and R$_{12}$ are independently selected from loweralkyl, aryl, arylalkyl, hydroxyalkyl, alkoxy, aminoalkyl, trialkylaminoalkyl, or heterocyclic.

Another still more preferred embodiment of the invention is a compound of formula I or II wherein n is 0, R is -C(O)$_2$-G wherein G is hydrogen or a carboxy protecting group, tetrazolyl or -C(O)-NHS(O)$_2$R$_{16}$ wherein R$_{16}$ is loweralkyl or

haloalkyl, Z is $-CH_2-$, R_1 is loweralkyl, alkenyl, heterocyclic (alkyl), aryloxyalkyl, arylalkyl, (N-alkanoyl-N-alkyl)aminoalkyl, alkylsulfonylamidoalkyl, phenyl, or alkoxyalkyl, R_2 is 1,3-benzodioxolyl, 1,4-benzodioxanyl, dihydrobenzofuranyl, benzofuranyl, 4-methoxyphenyl, dimethoxyphenyl, fluorophenyl or difluorophenyl and R_3 is R_4-C(O)-R_5- wherein R_5 is alkylene and R_4 is $(R_{11})(R_{12})N$- wherein R_{11} and R_{12} are independently selected from loweralkyl, aryl, arylalkyl, hydroxyalkyl, alkoxy, aminoalkyl, trialkylaminoalkyl, or heterocyclic.

A most highly preferred embodiment of the invention is a compound of formula I or II wherein n is 0, R is $-C(O)_2-G$ wherein G is hydrogen or a carboxy protecting group, Z is $-CH_2-$, R_1 is substituted or unsubstituted 4-methoxyphenyl, 4-fluorophenyl, 2-fluorophenyl, 4-methylphenyl, 4-trifluoromethylphenyl, 4-pentafluoroethylphenyl, 4-methoxymethoxyphenyl, 4-hydroxyphenyl, 4-ethylphenyl, 1,3-benzodioxolyl, 1,4-benzodioxanyl or dihydrobenzofuranyl wherein the substituent is selected from alkoxy, alkoxyalkoxy and carboxyalkoxy, R_2 is 1,3-benzodioxolyl, 1,4-benzodioxanyl, dihydrobenzofuranyl, benzofuranyl, 4-methoxyphenyl,

dimethoxyphenyl, fluorophenyl or difluorophenyl and R_3 is R_4-C(O)-R_5- wherein R_5 is alkylene and R_4 is $(R_{11})(R_{12})N$- wherein R_{11} and R_{12} are independently selected from loweralkyl.

Another most highly preferred embodiment of the invention is a compound of formula I or II wherein n is 0, R is $-C(O)_2-G$ wherein G is hydrogen or a carboxy protecting group, Z is $-CH_2-$, R_1 is substituted or unsubstituted 4-methoxyphenyl, 4-fluorophenyl, 2-fluorophenyl, 4-methylphenyl, 4-trifluoromethylphenyl, 4-pentafluoroethylphenyl, 4-methoxymethoxyphenyl, 4-hydroxyphenyl, 4-ethylphenyl, 1,3-benzodioxolyl, 1,4-benzodioxanyl or dihydrobenzofuranyl wherein the substituent is selected from alkoxy, alkoxyalkoxy and carboxyalkoxy, R_2 is 1,3-benzodioxolyl, 1,4-benzodioxanyl, dihydrobenzofuranyl, benzofuranyl, 4-methoxyphenyl, dimethoxyphenyl, fluorophenyl or difluorophenyl and R_3 is R_4-C(O)-R_5- wherein R_5 is alkylene and R_4 is $(R_{11})(R_{12})N$- wherein R_{11} is loweralkyl and R_{12} is aryl.

Another most highly preferred embodiment of the invention is a compound of formula I or II wherein n is 0, R is $-C(O)_2-G$ wherein G is hydrogen or a carboxy protecting group, Z is $-CH_2-$, R_1 is substituted or unsubstituted 4-methoxyphenyl, 3-

fluoro-4-methoxyphenyl, 3-fluorophenyl, 2-fluorophenyl, 3-fluoro-4-ethoxyphenyl, 4-methoxymethoxyphenyl, 1,3-benzodioxolyl, 1,4-benzodioxanyl or dihydrobenzofuranyl wherein the substituent is selected from loweralkyl, haloalkyl, alkoxy, alkoxyalkoxy and carboxyalkoxy, R_2 is substituted or unsubstituted 1,3-benzodioxolyl, 7-methoxy-1,3-benzodioxolyl, 1,4-benzodioxanyl, 8-methoxy-1,4-benzodioxanyl, dihydrobenzofuranyl, 4-methoxyphenyl, dimethoxyphenyl, fluorophenyl or difluorophenyl wherein the substituent is selected from loweralkyl, alkoxy and halogen and R_3 is R_6-$S(O)_2$-$N(R_{21})$-R_{10}- wherein R_{10} is alkylene, R_6 is loweralkyl, haloalkyl, alkoxyalkyl or haloalkoxyalkyl and R_{21} is loweralkyl, haloalkyl or alkoxyalkyl.

Another most highly preferred embodiment of the invention is a compound of formula I or II wherein n is 0, R is $-C(O)_2-G$ wherein G is hydrogen or a carboxy protecting group, Z is $-CH_2-$, R_1 is substituted or unsubstituted 4-methoxyphenyl, 3-fluoro-4-methoxyphenyl, 3-fluorophenyl, 2-fluorophenyl, 3-fluoro-4-ethoxyphenyl, 4-methoxymethoxyphenyl, 1,3-benzodioxolyl, 1,4-benzodioxanyl or dihydrobenzofuranyl wherein the substituent is selected from loweralkyl,

haloalkyl, alkoxy, alkoxyalkoxy and carboxyalkoxy, R_2 is substituted or unsubstituted 1,3-benzodioxolyl, 7-methoxy-1,3-benzodioxolyl, 1,4-benzodioxanyl, 8-methoxy-1,4-benzodioxanyl, dihydrobenzofuranyl, 4-methoxyphenyl, dimethoxyphenyl, fluorophenyl or difluorophenyl wherein the substituent is selected from loweralkyl, alkoxy and halogen and R_3 is R_4-C(O)-R_5- wherein R_5 is alkylene and R_4 is $(R_{11})(R_{12})N$- wherein R_{11} is alkyl and R_{12} is selected from aryl, aminoalkyl, trialkylaminoalkyl, and heterocyclic.

Another most highly preferred embodiment of the invention is a compound of formula I or II wherein n is 0, R is $-C(O)_2-G$ wherein G is hydrogen or a carboxy protecting group, Z is $-CH_2-$, R_1 is loweralkyl,alkenyl, heterocyclic (alkyl), aryloxyalkyl, aryalkyl, aryl, (N-alkanoyl-N-alkyl)aminoalkyl, or alkylsulfonylamidoalkyl, and R_3 is R_4-C(O)-R_5- wherein R_5 is alkylene and R_4 is $(R_{11})(R_{12})N$- wherein R_{11} and R_{12} are independently selected from alkyl, aryl, hydroxyalkyl, alkoxy, aminoalkyl, trialkylaminoalkyl, and heterocyclic, with the proviso that one or R_{11} and R_{12} is alkyl.

Another most highly preferred embodiment of the invention is a compound of formula I or II wherein n is 0, Z is $-CH_2-$,

and R_3 is R_4-C(O)-R_5- wherein R_4 is $(R_{11})(R_{12})N$- as defined therein and R_5 is alkylene.

Another most highly preferred embodiment of the invention is a compound of formula I or II wherein n is 0, Z is $-CH_2-$, R_1 is loweralkyl, and R_3 is R_4-C(O)-R_5- wherein R_4 is $(R_{11})(R_{12})N$- as defined therein and R_5 is alkylene.

Another most highly preferred embodiment of the invention is a compound of formula I or II wherein n is 0, Z is $-CH_2-$, R_1 is alkenyl, and R_3 is R_4-C(O)-R_5- wherein R_4 is $(R_{11})(R_{12})N$- as defined therein and R_5 is alkylene.

Another most highly preferred embodiment of the invention is a compound of formula I or II wherein n is 0, Z is $-CH_2-$, R_1 is heterocyclic (alkyl), and R_3 is R_4-C(O)-R_5- wherein R_4 is $(R_{11})(R_{12})N$- as defined therein and R_5 is alkylene.

Another most highly preferred embodiment of the invention is a compound of formula I or II wherein n is 0, Z is $-CH_2-$, R_1 is aryloxyalkyl, and R_3 is R_4-C(O)-R_5- wherein R_4 is $(R_{11})(R_{12})N$- as defined therein and R_5 is alkylene.

Another most highly preferred embodiment of the invention is a compound of formula I or II wherein n is 0, Z is $-CH_2-$,

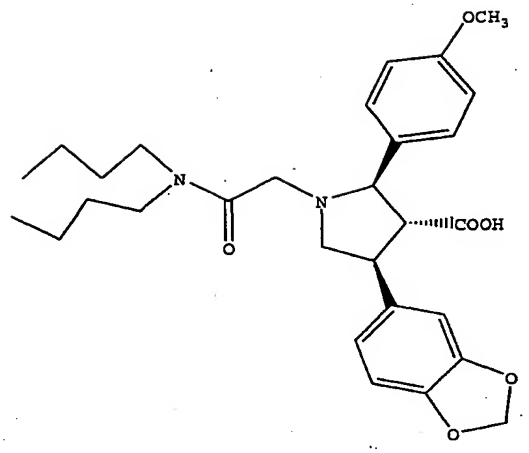
R_1 is arylalkyl, and R_3 is R_4-C(O)-R_5- wherein R_4 is $(R_{11})(R_{12})$N- as defined therein and R_5 is alkylene.

Another most highly preferred embodiment of the invention is a compound of formula I or II wherein n is 0, Z is $-CH_2-$, R_1 is aryl, and R_3 is R_4-C(O)-R_5- wherein R_4 is $(R_{11})(R_{12})$N- as defined therein and R_5 is alkylene.

Another most highly preferred embodiment of the invention is a compound of formula I or II wherein n is 0, Z is $-CH_2-$, R_1 is (N-alkanoyl-N-alkyl)aminoalkyl, and R_3 is R_4-C(O)-R_5- wherein R_4 is $(R_{11})(R_{12})$N- as defined therein and R_5 is alkylene.

Another most highly preferred embodiment of the invention is a compound of formula I or II wherein n is 0, Z is $-CH_2-$, R_1 is alkylsulfonylamidoalkyl, and R_3 is R_4-C(O)-R_5- wherein R_4 is $(R_{11})(R_{12})$N- as defined therein and R_5 is alkylene.

A particularly preferred compound of formula I is a compound of formula III, also known as ABT-627:



III

Compounds of formula I, II, and III may be synthesized by methods provided in commonly owned U.S. patent application Serial No. 09/048,955, filed March 27, 1998, U.S. patent application Serial No. 08/794,506, filed February 4, 1997, U.S. patent application Serial No. 08/600,625, filed February 13, 1996, U.S. patent application Serial No. 08/497,998, filed August 2, 1995, U.S. patent application Serial No. 08/442,575, filed May 30, 1995, U.S. patent application Serial No. 08/334,717, filed November 4, 1994, and U.S. patent application Serial No. 08/293,349, filed August 19, 1994, the disclosures of which are herein incorporated by reference.

Other suitable endothelin ET-A receptor antagonist may

be used, such as those disclosed in U.S. Patent Nos. 6,048,893, 6,017,951, and 5,998,468.

The term "inhibit" is defined to include its generally accepted meaning which includes preventing, prohibiting, restraining, and slowing, stopping or reversing progression, or severity, and holding in check and/or treating existing characteristics. The present method includes both medical therapeutic and/or prophylactic treatment, as appropriate.

The methods of the present invention are useful in men as well as in women. Preferably, however, the methods of the present invention are useful in men, more preferably men with prostate cancer.

The ability of the compounds of formula I, II, and III to treat cancers can be demonstrated according to the method described in J. Clin. Invest. 87 1867 (1991). Types of cancer includes primary cancer such as breast, prostate, lung, kidney, thyroid, myeloma, lymphoma, sarcoma, osteosarcoma, and ovarian.

The ability of the compounds of the invention to treat nociception can be demonstrated according to the method described in J. Pharmacol. Exp. Therap. 271 156 (1994).

The compounds of the present invention can be used in the

form of salts derived from inorganic or organic acids. These salts include but are not limited to the following: acetate, adipate, alginate, citrate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, camphorate, camphorsulfonate, digluconate, cyclopentanepropionate, dodecylsulfate, ethanesulfonate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, fumarate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxy-ethanesulfonate, lactate, maleate, methanesulfonate, nicotinate, 2-naphthalenesulfonate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, p-toluenesulfonate and undecanoate. Also, the basic nitrogen-containing groups can be quaternized with such agents as loweralkyl halides, such as methyl, ethyl, propyl, and butyl chloride, bromides, and iodides; dialkyl sulfates like dimethyl, diethyl, dibutyl, and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides, aralkyl halides like benzyl and phenethyl bromides, and others. Water or oil-soluble or dispersible products are thereby obtained.

Examples of acids which may be employed to form

pharmaceutically acceptable acid addition salts include such inorganic acids as hydrochloric acid, sulphuric acid and phosphoric acid and such organic acids as oxalic acid, maleic acid, succinic acid and citric acid.

Basic addition salts can be prepared *in situ* during the final isolation and purification of the compounds of formula I, or separately by reacting the carboxylic acid function with a suitable base such as the hydroxide, carbonate or bicarbonate of a pharmaceutically acceptable metal cation or with ammonia, or an organic primary, secondary or tertiary amine. Such pharmaceutically acceptable salts include, but are not limited to, cations based on the alkali and alkaline earth metals, such as sodium, lithium, potassium, calcium, magnesium, aluminum salts and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like. Other representative organic amines useful for the formation of base addition salts include diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like.

The compounds of formulas I, II and III are useful for

antagonizing endothelin in humans or other mammals. Total daily dose administered to a host in single or divided doses may be in amounts, for example, from 0.001 to 1000 mg/kg body weight daily and more usually 0.1 to 100 mg/kg for oral administeration or 0.01 to 10 mg/kg for parenteral administeration. Dosage unit compositions may contain such amounts of submultiples thereof to make up the daily dose.

Pharmaceutical formulations may be prepared by procedures known in the art. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administeration.

It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administeration, route of administeration, rate of excretion, drug combination, and the severity of the particular disease undergoing therapy.

The compounds of the present invention may be administered orally, buccally, parenterally, sublingually, by inhalation spray, rectally, or topically in dosage unit

formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles as desired. Topical administeration may also involve the use of transdermal administeration such as transdermal patches or iontophoresis devices. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection, transcutaneous, intradermal, or infusion techniques.

Injectable preparations, for example, sterile injectable aqueous or oleagenous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-propanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Suppositories for rectal administeration of the drug can be prepared by mixing the drug with a suitable nonirritating excipient such as cocoa butter and polyethylene glycols which are solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum and release the drug.

Solid dosage forms for oral administeration may include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound may be admixed with at least one inert diluent such as sucrose lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e.g., lubricating agents such as magnesium stearate. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administeration may include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions may also comprise adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents.

The compounds of the present invention can also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain, in addition to a compound of the present invention, stabilizers, preservatives, excipients, and the like. The preferred lipids are the phospholipids and phosphatidyl cholines (lecithins), both natural and synthetic.

Methods to form liposomes are known in the art. See, for example, Prescott, Ed., Methods in Cell Biology, Volume XIV, Academic Press, New York, N.Y. (1976), p. 33 et seq.

A representative solid dosage form, for example, a tablet or a capsule, comprises:

Compound of the invention:	35% w/w
Starch, Pregelatinized, NF	50% w/w
Microcrystalline Cellulose, NF	10% w/w
Talc, Powder, USP	5% w/w

While the compounds of the invention can be administered

as the sole active therapeutic agent, they can also be used in combination with one or more co-therapeutic agents, such as anticancer drugs or methods including, but not limited to, hormonal agents, such as leuprolide (Lupron®); gonadorelin antagonists, such as goserelin (Zoladex®) and abarelix; bicalutamide; nilutamide; flutamide; vitamin D; vitamin D analogues; estrogen and estrogen analogues, such as diethylstibestrol; prednisone; hydrocortisone; ketoconazole; cyproterone acetate; progesterone; 5-alpha reductase inhibitors, such as finasteride; bone-seeking radionuclides, such as samarium (Quadramet®), strontium (Metastron®), and ^{186}rhenium; external beam radiation, including three dimensional conformal radiation; brachytherapy, which is the implantation of radioactive seeds directly into the prostate; monoclonal antibodies such as trastuzumab (Herceptin®); anti-angiogenic agents such as thrombospondin peptide or kringle 5; matrix metalloproteinase inhibitors; farnesyl transferase inhibitors; lycopenes; urokinase; plasminogen activator inhibitors; plasminogen activator receptor blockers; apoptosis inducers; selective and non-selective alpha blockers; platinum agents, such as cis-platinum and carbo-platinum; taxane class agents, such as docitaxil and paclitaxil; estramustine;

gemcytabine; adriamycin; doxorubicin; daunorubicin; mitoxantrone; vinblastine; vincristine; capecitabine; irinotecan; topotecan; 5-fluorouracil; interferons; cytoxan; methotrexate; cytokines, such as IL-2; PPAR agonists, such as thiazolidine diones; retinoid-type agents, 5-lipooxygenase inhibitors, such as zyfo (Zilueton®), COX-2 inhibitors; gene-therapy based therapeutics, including sense and anti-sense genes; cholesterol lowering drugs, such as lovastatin, pravastatin, and simvistatin; bisphosphonates; osteoprotegrin; and antibodies, both monoclonal and polyclonal; antibody-coupled radionucleotides; antibody-coupled cytotoxic agents; antibody-coupled radionucleotides; viral-vector delivered agents; vaccines directed at protein, carbohydrate, or nucleic acid targets; aminoglutethimide; and suramin.

These combinations can be administered as separate compositions or as a single dosage form containing both or all agents. When administered as a combination, the therapeutic agents can be formulated as separate compositions, which are given at the same time or different times, or the therapeutic agents can be given as a single composition.

In addition, the compounds invention can be used in

combination with one or more co-therapeutic agents which impede net bone loss, such as estrogens, bisphosphonates, and estrogen receptor modulators, such as raloxifene, and calcitonin.

The compounds of the invention can additionally be administered in combination with surgery, such as radical prostatectomy, cryotherapy, transurethral resection of the prostate as an adjuvant, and the like, or prior to surgery as a neoadjuvant agent.

The current major diseases or conditions of bone which are of public concern include, but are not limited to, post-menopausal osteoporosis, ovariectomy patients, senile osteoporosis, patients undergoing long-term treatment of corticosteroids, side effects from glucocorticoid or steroid treatment, patients suffering from Cushings's syndrome, gonadal dysgenesis, periarticular erosions in rheumatoid arthritis, osteoarthritis, Paget's disease, osteohalisteresis, osteomalacia, hypercalcemia of malignancy, osteopenia due to bone metastases, periodontal disease, hyperparathyroidism, osteroperosis from Lupron therapy, and starvation. All of these conditions are characterized by bone loss, resulting from an imbalance between the degradation of bone (bone

resorption) and the formation of new healthy bone. This turnover of bone continues normally throughout life and is the mechanism by which bone regenerates. However, the conditions stated above will tip the balance towards bone loss such that the amount of bone resorbed is inadequately replaced with new bone, resulting in net bone loss.

Examples

Studies were performed on male subjects with asymptomatic hormone refractory prostate cancer with rising PSA levels and on male subjects with symptomatic hormone refractory prostate cancer with rising PSA levels and pain. Subjects in the phase II studies had castrate levels of testosterone, either due to pharmacologic intervention, via leuprolide (Lupron®) or goserelin (Zoladex®), or via surgical castration. Subjects received ABT-627 or placebo. The following tests were conducted:

ABT-627 was formulated in 2.5 and 10 mg doses. An oral liquid formulation of ABT-627 was also prepared as follows: 1 mg/ml ABT-627, 50% glycerin, 14% alcohol, and water. Matching placebos were also provided.

A number of recognized or putative biochemical markers of

disease progression have been used to monitor treatment of individuals with prostate cancer. Among these markers are serum Prostate Specific Antigen (PSA), serum acid Phosphatase, Interleukin-6, and Chromagranin-A. As currently accepted, favorable treatment is marked by a decrease or slower rate of increase for PSA, acid phosphatase, and Interleukin-6, while a favorable response is marked by an increase in Chromagranin-A.

Serum samples were obtained from subjects during treatment with the ET antagonist ABT-627 in order to determine PSA, acid phosphatase, IL-6, and Chromagranin-A values.

Prostate Specific Antigen Level Assay

The effect of ABT-627 administeration on prostate specific antigen (PSA) levels in human subject serum samples was determined using the procedure described in the Chiron Diagnostics ACS: Centaur PSA2 Assay. This assay is a two-site sandwich immunoassay which uses direct chemiluminescense and constant amounts of two antibodies. The first antibody, the Lite Reagent, is an affinity purified polyclonal sheep anti-PSA antibody labeled with acridinium ester. The Lite Reagent is purchased as a 5.0 mL reagent pack comprising the polyclonal sheep anti-PSA antibody (3.1 μg) in buffered saline

with sodium azide (0.1%). The second antibody, the Solid Phase, is a monoclonal mouse anti-PSA antibody covalently coupled to paramagnetic particles. The Solid Phase is purchased as a 25.0 mL reagent pack comprising the covalently coupled monoclonal mouse anti-PSA antibody (316 µg) in buffered saline with sodium azide (0.1%). The assay was performed at Quintiles Laboratories (Smyrna, GA) using Chiron Diagnostics ACS: Centaur® Automated Chemiluminescence Systems.

Briefly, a subject population was treated with a placebo or 2.5 mg or 10 mg of ABT-627. Blood samples were collected, allowed to adequately clot, centrifuged at 1000 x g for 15-20 minutes, and stored at -20 °C if not assayed within 48 hours. A cuvette was charged sequentially with serum, Lite Reagent (50 µL), and Solid Phase (250 µL). The resulting mixture was incubated for 7.5 minutes at 37 °C, separated, and treated with the solution of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction. A direct relationship exists between the amount of PSA present in the patient sample and the RLU's (relative light units) detected. As shown by the area under the curve (AUC) in Figure 2, the rate of increase of PSA in the serum samples decreases after the adminsteration of ABT-627, demonstrating the effectivness of ABT-627 as an

agent for treating prostate cancer.

Acid Phosphatase Levels

The effect of ABT-627 administeration on Acid Phosphatase levels in human subject serum samples was determined at Quintiles Laboratories using the chemical test described in Sigma Diagnostics Acid Phosphatase (ACP) Procedure No. 435. The enzyme Acid Phosphatase (ACP) catalyzes the hydrolysis of alpha-naphthyl phosphate to alpha-naphthol and inorganic phosphate. The alpha-naphthol immediately reacts with fast red TR salt to produce a yellow chromophore with an absorbance maximum at 405 nm. The rate of increase in absorbance at 405 nm is directly proportional to ACP activity in the sample. ACP activity was determined in the presence and absence of L-tartrate, the difference being attributed to prostatic acid phosphatase activity.

Briefly, a subject population was treated with a placebo or 2.5 mg or 10 mg of ABT-627. Blood samples were collected, allowed to adequately clot, centrifuged at 1000 x g for 15-20 minutes, and stored at -20 °C if not assayed within 48 hours. Assays were performed on a Hitachi Spectrophotomer. A cuvette was charged sequentially with ACP reagent (1 mL), prepared as

described in the assay protocol, and serum (0.1 mL). The mixture was agitated and incubated for 5 minutes, and an absorbance (A) at 405 nm (versus water as a reference) was read to provide an initial absorbance. The mixture was incubated for another 5 minutes, and a second absorbance was read to provide a final absorbance. A change A/5 minute value was obtained by subtracting the initial absorbance from the final absorbance and was used to calculate total ACP activity.

To provide the tartrate-resistant acid phosphatase activity, the above procedure was repeated with the addition of ACP tartrate reagent (0.01 mL) to the cuvette containing the ACP reagent and mixing before adding the serum. Prostatic acid phosphatase activity was calculated by subtracting the the tartrate-resistant acid phosphatase activity from the ACP activity. As shown shown by the (AUC) in Figure 7, the rate of increase and the average change from baseline for acid phosphatase was decreased in those subjects treated with ABT-627, again demonstrating the effectivness of ABT-627 as an agent for treating prostate cancer.

Chromagranin-A Levels